SCIENTIFIC PRECIS (Abstract)

This is a Phase I/II clinical trial of *ex vivo* hematopoietic stem cell (HSC) gene therapy for X-linked severe combined immunodeficiency (XSCID). XSCID results from defects in the *IL2RG* gene encoding the common gamma chain (gc) shared by receptors for Interleukin 2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21. XSCID patients generally lack T-lymphocytes and NK cells, and their B-lymphocytes fail to make essential antibodies. XSCID is fatal in infancy without immune reconstitution, such as by allogeneic bone marrow transplantation (BMT). However, many transplanted patients achieve only partial immune reconstitution, and consequently have recurrent infections, autoimmunity and/or and poor growth. Recent successful retroviral gene therapy instead of BMT for infants with XSCID [1] indicates that *ex vivo* gene therapy can provide clinical benefit to XSCID patients, although leukemia occurred in two of the treated patients indicating that there are significant risks with gene therapy.

We will enroll six older XSCID patients (2-20 years-old; ≥15 kg body weight), who have had attempted BMT, but who have persistent T-lymphocyte and B-lymphocyte impairments that compromise their quality of life. Prior to enrollment, each case will be presented for review and risk/benefit assessment by the IRB and regulatory agencies. These patients will have had autologous CD34+ HSC mobilized by treatment with granulocyte colony stimulating factor (G-CSF), collected from peripheral blood by apheresis, immune selected and cryopreserved in sufficient numbers to achieve entry criteria ($\geq 1.0 \times 10^6$ CD34+ HSC/kg body weight). HSC procurement will be conducted under a separate, approved and active NIH protocol, 94-I-0073, "Recruitment of peripheral blood hematopoietic progenitors by granulocyte colony stimulating factor [G-CSF]" [Harry L. Malech, PI].

Autologous CD34+ HSC will be subjected to four daily transductions *ex vivo* with the gibbon ape leukemia virus (GALV) envelope-pseudotyped, replication-defective, murine onco-retrovirus vector, MFGS-gc that encodes the common gamma chain. Transductions will occur in flexible gas-permeable plastic containers using serum-free medium supplemented with 1% human serum albumin and five recombinant growth factors (50 ng/ml Flt3-L, 50 ng/ml SCF, 50 ng/ml TPO, 25 ng/ml IL-6, and 5 ng/ml IL-3). Each subject will receive a single infusion of transduced HSC. Subjects will be monitored for safety and efficacy; the latter evidenced by new development of autologous transduced lymphocytes with functional gc. Study endpoints are (1) efficient and safe clinical-scale transduction of HSC from post-BMT XSCID subjects; (2) administration of transduced HSC to six subjects; and (3) 3 year follow up of treated subjects to monitor vector sequence distribution, gc expression in hematopoietic lineages, and lymphocyte numbers and function; as well as general health and immune status.